New Challenge to Parasite Immunology

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**Recent progress on transmission-blocking vaccine development of vivax malaria**

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**Introduction**

Malaria remains a leading cause of high morbidity and mortality in human populations, particularly in sub-Saharan Africa. The causative agents of human malaria are four species of protozoan parasites belonging to the genus *Plasmodium*. Although *Plasmodium falciparum* is responsible for the highest mortality rate among the four species of human malaria parasites, vivax malaria is responsible for the most recurrent form of malaria, causing high morbidity including repeated attack for millions of people in tropical and subtropical countries outside of sub-Saharan Africa (Mendis, *et al* 2001). Although significant efforts have been made to control malaria through several strategies such as the development of effective anti-malaria drugs and the use of insecticide-impregnated mosquito nets, current vector control and malaria chemotherapy are becoming ineffective due to the spread of multidrug-resistant parasites and insecticide resistant mosquitoes (Greenwood and Mutabingwa 2002, Harinasuta, *et al* 1965, Rieckmann, *et al* 1989, Roberts and Andre 1994). In response to the complex life cycle of malaria parasites and the discrete nature of effective immune responses to each developmental stage, considerable efforts have been made to develop effective vaccines targeting each stage (Richie and Saul 2002). There are three developmental stages of malaria parasites against which vaccines are being developed. Vaccines against sporozoites and liver stage parasites would prevent infection in a human host; those against the asexual blood stage parasites would stop the propagation of the parasites in the blood. Although not providing direct protection for vaccinees, vaccines targeting antigens expressed on the surface of the sexual (gametocyte, gamete, zygote, ookinete) stages of malaria
parasites are considered one promising strategy for malaria control (Carter 2001, Kaslow 1997, Stowers and Carter 2001). Such vaccines, called transmission-blocking vaccines (TBVs), induce antibodies in the human host that inhibit the parasite’s development within mosquito midguts and consequently completely block parasite transmission to another human host. Although TBVs are thought to be effective in interrupting parasite transmission, TBVs would also protect other vaccines or drugs against the spread of resistant parasites, greatly prolonging their effective life (Stowers and Carter 2001, Tsuboi, et al In Press).

In this review article, we describe the recent progress for *P. vivax* TBV development.

**Target antigens of transmission-blocking vaccine**

Target antigens for a transmission-blocking vaccine against *P. falciparum* are the ookinete surface proteins of *P. falciparum*, Pfs25 and Pfs28 (Barr, et al 1991, Duffy and Kaslow 1997, Kaslow, et al 1988). Their homologues have been cloned from other species of malaria parasites (Kaslow, et al 1989, Paton, et al 1993, Tachibana, et al 2001, Tsuboi, et al 1997a, Tsuboi, et al 1997b). They all have conserved structures composed of four tandem epidermal growth factor (EGF)-like domains, anchored to the parasite surface by a glycosylphosphatidylinositol moiety. To search for P25 and P28 orthologues in *P. vivax*, we aligned the DNA sequences of the eight known proteins in the P25 group (Pfs25, Pgs25, Pys25, Pbs25) and in the P28 group (Pfs28, Pgs28, Pys21, Pbs21) of zygote/ookinete surface proteins. Regions of highest identity were used to design degenerate PCR oligonucleotides. Genomic DNA from the Salvador (Sal) I strain of *P. vivax* were used as PCR templates. Using this approach, Pvs25 and Pvs28 genes were successfully cloned (Tsuboi, et al 1998) (Fig. 1). This success was ten years behind the gene cloning of Pfs25 from *P. falciparum* (Kaslow, et al 1988).

**Immunogenicity of vivax transmission-blocking vaccine and their efficacy on Sal I parasite**

The recombinant Pvs25 and Pvs28 were expressed in yeast, where posttranslational protein modifications can occur, because a proper conformation is required to induce transmission-blocking antibodies (Duffy, et al 1993). The mass spectroscopic analyses of the recombinant molecules that we produced suggest that all cysteines in recombinant Pvs25 formed disulfide bonds. These molecules also show a shift in apparent molecular mass between reduced and nonreduced SDS-PAGE. Furthermore, vaccination with these proteins induced biologically
active antibodies in terms of recognition of parasite protein on the surface of cultured ookinetes (Hisaeda, et al 2000) (Fig. 2). Mice vaccinated with the yeast-produced Pvs25 and Pvs28 adsorbed to aluminum hydroxide (alum) developed strong antibody responses against the immunogens. Recombinant Pvs25 is highly immunogenic in all strains of mice tested and induced both T- and B-cell responses. For recombinant Pvs28 the results were very similar, with the exception of mice with an \( H-2^b \)-haplotype, which were unable to respond. This failure of Pvs28 to induce immune responses in \( H-2^b \)-bearing mice was likely due to the failure of major histocompatibility complex class II molecules (specifically the \( I-A^b \) molecule) of this strain to present T-cell epitopes from Pvs28. Hence, vaccination with Pvs28 failed to activate helper T cells and consequently B-cell antibody production. This major histocompatibility complex-linked unresponsiveness is a problem that will need to be addressed in future vaccine development (Hisaeda, et al 2000). Then, the transmission-blocking activity of these mouse antisera was analyzed. The assay system used was based on membrane feeding to mosquitoes (Tsuboi, et al In Press) using peripheral blood obtained from a chimpanzee infected with the \( P. vivax \) Sal I strain (identical to that from which the genes encoding Pvs25 or Pvs28 were cloned) (Sullivan, et al 1996). Four species of susceptible
Fig. 2 Recombinant Pvs25 and Pvs28 induce specific antibodies, which recognize native proteins of *P. vivax*.

Anopheline mosquitoes developed oocysts in their midguts after ingestion of a *P. vivax*-infected blood meal mixed with control serum through a membrane-feeding apparatus. In contrast, oocyst formation was dramatically suppressed when anti-Pvs25 or -Pvs28 antisera from CAF1 mice were mixed with the infected blood. Anti-Pvs25 antiserum completely prevented oocyst formation in all mosquitoes. Of all mosquitoes fed with anti-Pvs28 antiserum, only one became infected, and that with a single oocyst. One of the representative results was presented in Fig. 3. Blocking by anti-Pvs25 antiserum was much more effective at 1:8 or 1:32 dilution compared with that by anti-Pvs28 antiserum (Hisaeda, *et al* 2000).

Based on these data, we have elected in the first instance to concentrate our vaccine development efforts on recombinant Pvs25. It gives a higher yield in in vitro expression systems, an important consideration for a vaccine to be used in developing countries. As a recombinant protein, it is better characterized, with no apparent posttranslational modifications and an appropriate secondary structure. The antigenic variation of Pvs25 in field isolates appears to be
Fig. 3  Complete transmission-blocking activity of mouse antisera against Pvs25 and Pvs28 on *P. vivax* Sal I strain.


**Efficacy of Sal I based vaccine on natural parasite population**
The question whether the antisera to the TBV antigens derived from Sal I parasite sequences inhibit transmission of field-isolated parasites has been considered to be critical for the TBV development. Thus, we tested the immunogenicity of recombinant Pvs25 and Pvs28 in mice with alum as an adjuvant and found that mice, when immunized three times with 50 µg of immunogen, induced comparable levels of serum antibody responses and transmission-blocking immunity against field isolates, as compared with the efficacy obtained from the mouse immune sera against the Sal I strain (Hisaeda, et al 2000). For most (90%) human isolates, sera from mice immunized using alum as an adjuvant showed complete inhibition of oocyst development (unpublished results). Transmission-blocking experiments conducted in this study clearly demonstrate that antisera to recombinant Pvs25 and Pvs28, produced based on the gene sequence of the *P. vivax* Sal I strain, could recognize the corresponding molecules expressed by field-isolated parasites. Moreover, the antisera showed potent transmission-blocking activities on the natural parasite populations, supporting the concept that TBVs could overcome the problem of antigenic polymorphisms. One of the reasons why the Sal I based vaccine was also effective on the polymorphic target antigens is because almost all the amino acid substitutions detected previously (Tsuboi, et al 1998) were conservative substitutions. Thus, the tertiary structure and epitopes of transmission-blocking antibodies may be conserved. In addition to this evidence, the low substitution frequencies, the conservative nature of the substitutions and the likelihood that these have not been selected as a result of immune pressure (Taylor, et al 2000), all suggest that antigenic diversity will not be a major issue for Pvs25 and Pvs28 based TBVs.

Significant efforts have been made to control malaria through several strategies such as the development of effective anti-malaria drugs and the use of insecticide-impregnated mosquito nets in highly endemic areas. However, emergence of drug-resistant parasites and insecticide-resistant mosquitoes has made chemotherapy and vector control difficult (Breman 2001). A TBV will not prevent the emergence of drug resistant mutants, but prevent the spread of drug resistant parasites throughout the human population in endemic areas. Thus, even in areas where a TBV may not have sufficient impact to reduce the parasite prevalence, it is expected that is would have significant impact on spread of escape mutants. Although it would be difficult to introduce a transmission-blocking vaccine for this purpose alone, we anticipate that the problems observed with the spread of drug resistant malaria will be mimicked by the spread of vaccine-resistant parasites. Recently, there is evidence that the vaccine induced selection pressure exerted on the parasite population in the field trial of an erythrocytic stage malaria vaccine in Papua New Guinea (Genton, et al 2002). This result strongly argues for developing vaccines comprising conserved antigens and/or multiple components covering all dominant allelic types.
Therefore, incorporation of a TBV with vaccines targeting the other stages may be an important strategy to preserve their effective life. Accordingly, for many malarious regions outside of Africa, development of effective TBV will require coverage against both *P. falciparum* and *P. vivax*. At this moment, Phase I clinical trials by using yeast-produce Pvs25 as vaccine has started, and formulation of PfS25 molecule is in preparation (Allan Saul, personal communication).

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**References**


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